

Theoretical Modeling of the Heme Group with a Hybrid QM/MM Method

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ABSTRACT: The quality of the results obtained in calculations with the hybrid QM/MM method IMOMM on systems where the heme group is partitioned in QM and MM regions is evaluated through the performance of calculations on the 4-coordinate [Fe(P)] (P = porphyrin), the 5-coordinate [Fe(P)(1 – (Me)Im)] (Im = imidazole) and the 6-coordinate [Fe(P)(1 – (Me)Im)(O₂)] systems. The results are compared with those obtained from much more expensive pure quantum mechanics calculations on model systems. Three different properties are analyzed—namely, the optimized geometries, the binding energies of the axial ligands to the heme group, and the energy cost of the biochemically relevant out-of-plane displacement of the iron atom. Agreement is especially good in the case of optimized geometries and energy cost of out-of-plane displacements, with larger discrepancies in the case of binding energies. © 2000 John Wiley & Sons, Inc. *J Comput Chem* 21: 282–294, 2000

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Introduction

The heme group occupies a prominent position in biochemistry. It is in the active center of a number of very relevant proteins. Among these, there are the oxygen transport proteins hemoglobin and myoglobin,¹ as well as enzymes involved in catabolism as peroxidases,² catalases, oxidases,³ and cytochromes.⁴ Furthermore, other very relevant groups are structurally related to heme. The replacement of Fe by Mg leads to chlorophyll;⁵ and replacement of the metal by other transition metals coupled with modifications in the aromatic ring, leads to species as vitamin B₁₂⁶ and cofactor F-430.⁷ It is, thus, no wonder that the study of bioinorganic models of the heme group has been a focal point of experimental bioinorganic chemistry for years.⁸

Experimental characterization of the reactivity of the heme group appears, however, as challenging. Its versatility, which is probably the key to its biochemical activity, makes experimental isolation of active species very difficult. Because of that, the heme group is an appealing target for theoretical methods. There are indeed a number of studies. One can cite in this concern the early works by Rohmer, Dedieu, and coworkers on the characterization of the electronic state of Fe(P) (P = porphyrin) complexes,⁹ where they could predict an electronic structure that was later on proved by experiment.¹⁰ There are also a number of issues where theoretical studies are having an impact on the ongoing discussion, as the real position of the CO group in [Fe(P)(imidazole)(CO)] complexes,^{11,12} the role of distal and proximal histidines on the binding of oxygen in hemoglobin,^{13–15} or structural aspects of the binding of dioxygen and other ligands to heme substrates.^{16,17} The amount of information that can be obtained from the calculations is, however, seriously limited by the size of the heme group itself, with its 24-s period atoms, which poses a very serious strain in the computational demand, and that has allowed only recently the appearance of theoretical studies on reactivity.^{18–20}

Hybrid QM/MM methods are currently emerging as a powerful tool for the quantitative study of large systems. In these methods, the system is partitioned in different regions, with the higher level method being applied only to the region where it is required. In this way the computational effort of the calculation can be dramatically reduced, and, if the partition is appropriately chosen, the results are practically unchanged. We have already applied one

of these methods, IMOMM,²¹ to a number of transition metal systems with success.²²

Application of this kind of methods to the modeling of heme groups raises the question of the validity of introducing a QM/MM partition *within* the porphyrin ring. The fact that the four nitrogen donor atoms of the porphyrin are part of a large aromatic ring has obviously two types of effects on the complex. One of them is the existence of a quite rigid framework, which, among other things, precludes distortions towards a tetrahedral arrangement. This effect can be well reproduced in IMOMM calculations. The second effect concerns the electronic possibilities of the porphyrin ring as an electronic reservoir where the occupied and empty orbitals are relatively close. This effect will be poorly reproduced in IMOMM calculations. Some available data on pure QM calculations with [Fe(NH(CH)₃NH)₂] models,²³ as well as a preliminary IMOMM study on Fe(P)(imidazole)(O₂) look nevertheless promising.²⁴

The current article presents a systematic study of the performance of the IMOMM method on a series of systems involving the heme group. These are the 4-coordinate [Fe(P)] system, the 5-coordinate [Fe(P)(Im)] system, and the 6-coordinate [Fe(P)(Im)(O₂)] system. For each of these systems, a series of properties are studied, the focus being in the comparison of the IMOMM results with those of much more expensive full QM calculations. After the computational details, a section follows on each of the three coordination modes. An additional section analyzes the results of a pure QM optimization of the QM region of the IMOMM calculation. Afterwards, the computational level used for the QM part is briefly discussed, and a final section collects the conclusions.

Computational Details

Pure quantum mechanics calculations are performed with the Gaussian 94 program.²⁵ Most calculations are carried out at the unrestricted Becke3LYP level,²⁶ with some test calculations being carried at the unrestricted Hartree–Fock (UHF) level. Several basis sets are applied. In all of them a quasi-relativistic effective core potential (ECP) replaces the 10 innermost electrons of Fe.²⁷ The basis set for Fe is always the valence double- ζ contraction labeled LANL2DZ associated to this ECP.^{25,27} In basis set I, used in all calculations unless otherwise stated, the basis set is 6-31G(d) for N, O;^{28,29} and STO-3G for C, H.³⁰ Five other basis sets are constructed through modifications in the description of

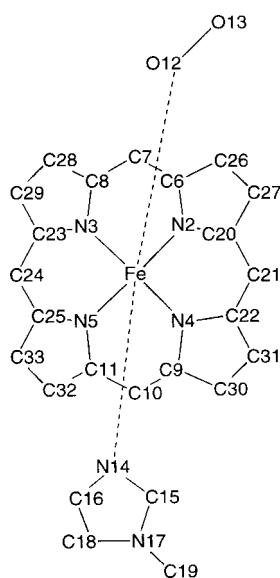
these atoms. Basis set II uses a STO-3G description for N, O, C, H.³⁰ Basis set III places 6-31G on N, O;²⁸ and STO-3G on C, H. In basis set IV, the 6-31G set is applied on N, O, C, H. Basis set V uses 6-31G(d) for N, O; and 6-31G for C, H. Finally, basis set VI consists of a 6-31G(d) description for N, O, C, H. Basis sets II to VI are only applied in the section devoted to evaluation of basis set quality. All geometry optimizations in the pure QM calculations are complete, with no symmetry restrictions. Spin contamination, measured through the expectation of S^2 , was in all cases small.

IMOMM calculations were carried out with a program built from modified versions of the two standard programs Gaussian 92/DFT³¹ and mm3(92).³² The quantum mechanics region is defined by atoms with labels inferior to 15 in Scheme 1. Thus, the heme group is modeled in the quantum region as $[\text{Fe}(\text{NH}(\text{CH})_3\text{NH})_2]$, as in previous QM calculations,²³ and the imidazole as NHCH_2 , with the dioxygen being introduced as such. The rest of the system constitutes the molecular mechanics region. The introduction of this QM/MM partition within the porphyrin ring has the inconvenient of downgrading the symmetry of the 4-coordinate system from D_{4h} to D_{2h} , but is in our opinion the best alternative. Other partitions with a smaller QM region could hardly keep the four equivalent sp^2 nitrogen atoms together with the dianionic nature of the porphyrin. The use of larger QM regions, apart from the associated increase in computational cost, is limited by the requirement of the IMOMM method, at least in its original formulation,²¹ that

no direct chemical bonds can be put between set 3 atoms, those connecting the QM and MM regions. This means that if, for instance, atoms C20 to C25 (Scheme 1) where introduced in the QM region, then the whole porphyrin, with atoms C26 to C31, would have to be included, because of the existence of bonds like that between C26 and C27. A final comment on the QM/MM partition concerns the imidazole ring. C15 and not C16 (Scheme 1) was included in the QM region because in the most simple Lewis structure of imidazole, the double bonds are between N14—C15 and C16—C18.

The QM part of the IMOMM calculation uses the same methods and basis sets described above for the pure QM calculations. The MM calculations use the mm3(92) force field.³³ Van der Waals parameters for the iron atom are taken from the UFF force field,³⁴ and torsional contributions involving dihedral angles with the metal atom in terminal position are set to zero. MM stretching and bending terms involving Fe are set to zero by the IMOMM method in the current QM/MM partition, and because of that no MM parameters are required for them. All geometrical parameters are optimized except the bond distances between the QM and MM regions of the molecules. The frozen values are 1.019 Å (N—H), 1.101 Å (C—H) in the QM part; and 1.378 Å (N—C, porphyrin ring), 1.332 Å (C—C), 1.414 Å (N—C, imidazole ring) in the MM part.

Atom numbering is that shown in Scheme 1. The molecule is oriented in such a way that the porphyrin ring is in the xy plane, and the projections of the Fe—N bonds lie approximately on the bisectors of the x and y axis.



SCHEME 1.

The 4-Coordinate [Fe(P)] System

The heme group as such has little direct application in biochemistry, but it is a natural starting point for both the experimental and the theoretical study. The crystal structures of a number of derivatives of heme have been reported, with different substituents in the ring. Unfortunately, the simplest model, with all substituents being hydrogen, has not been reported. Because of this, comparison will be made with a species containing some substituents. In particular, we have chosen $[\text{Fe}(\text{TPP})]$ (TPP = *meso*-tetraphenylporphyrin).¹⁰ The electronic state of this particular molecule is well known experimentally to correspond to a triplet ($S = 1$). X-ray studies of electron distribution have even allowed the experimental assignment of the electronic state.¹⁰ This assignment has also been

confirmed by recent theoretical studies.³⁵ In the ground state, the distribution of the electrons in the d orbitals of iron is the following: $d_{x^2-y^2}$, d_{z^2} , doubly occupied; d_{xz} , d_{yz} , singly occupied; d_{xy} , empty. Calculations are done only on this electronic state.

Calculations have been carried out on the [Fe(P)] model system at both the pure Becke3LYP and the IMOMM(Becke3LYP:MM3) computational levels. The optimized geometry from the IMOMM calculation is presented in Figure 1, and selected structural parameters of both calculations and experiment are collected in Table I. One first comment concerns the symmetry of the optimized geometry. Although all calculations were carried out without symmetry restrictions, the resulting geometries happen to have high symmetry. This is D_{4h} for the pure QM calculation and D_{2h} for the hybrid QM/MM calculation. Both computed geometries are planar (with an out of plane displacement of the atoms of 0°) and the difference in the symmetry of both geometries stems obviously from the partition in two different regions introduced in the IMOMM calculation. The contrast between the computed planar structures and the C_1 , nearly S_4 , symmetry of the experimental geometry was less expected. We attribute this difference to the fact that while the calculations are carried out on a gas phase [Fe(P)], experimental results are on a crystal structure of [Fe(TPP)]. The distortion from planarity in the experimental structure can be due to the presence of the phenyl groups,³⁶ or to packing effects.³⁷ Clarification of the origin of distortion from planarity in the experimental structure would require a further analysis, and is not the goal of this work.

Apart from the planarity, reflected in the average displacement out of the plane of the 24 atoms of the porphyrin ring, other data in Table I deserve comment. The agreement in bond angles between both computed geometries and the X-ray structure is excellent, with discrepancies always smaller

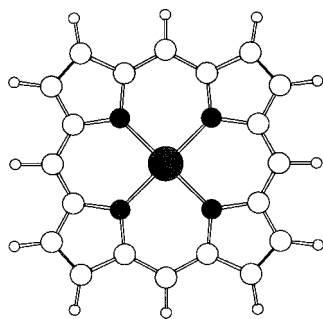


FIGURE 1. Optimized IMOMM(Becke3LYP:MM3) geometry of the 4-coordinate complex [Fe(P)].

TABLE I. Selected Geometrical Parameters (Å and Degrees) from the Geometry Optimization of [Fe(P)] with the Pure Becke3LYP and with the IMOMM(Becke3LYP:MM3) Methods.

	Experiment	Pure QM	QM/MM
Fe—N2	1.966	2.016	1.940
N2—C6	1.378 ^a	1.397	1.362
N2—C20	1.378 ^a	1.397	1.378 ^b
C6—C7	1.395 ^a	1.402	1.401
C6—C26	1.439 ^a	1.459	1.438 ^b
C20—C21	1.395 ^a	1.402	1.336
C20—C27	1.439 ^a	1.459	1.345
C26—C27	1.365 ^a	1.367	1.333
Fe—N2—C6	127.2 ^a	127.4	128.7
Fe—N2—C20	127.2 ^a	127.4	126.7
N2—Fe—N3	90.0 ^a	90.0	89.5
N2—Fe—N4	90.0 ^a	90.0	90.5
N2—C6—C7	125.3 ^a	125.5	126.9
N2—C6—C26	110.6 ^a	110.4	109.0
N2—C20—C21	125.3 ^a	125.5	125.4
N2—C20—C27	110.6 ^a	110.4	111.5
Fe-plane ^c	0.000	0.000	0.000
RMS displ. ^d	0.237	0.000	0.000

Experimental data on the [Fe(TPP)] system are also provided for comparison. Labeling of atoms is that from Scheme 1.

^a Average values.

^b Frozen in calculation.

^c Distance of the iron atom to the mean plane of the porphyrin ring.

^d Average out of plane displacement of the 24 atoms of the porphyrin ring.

than 2° . Discrepancies in bond distances are larger in a number of cases. Some of them were, nevertheless, to be expected. This is the case of the C20—C21, C20—C27, and C26—C27 distances. These distances have values of 1.395, 1.439, and 1.365 Å, respectively, in X-ray; 1.402, 1.459, and 1.367 Å, respectively, in Becke3LYP; and 1.336, 1.345, and 1.333 Å, respectively, in IMOMM. Agreement between experiment and pure QM is good (within 0.02 Å), while the discrepancy with IMOMM is up to 0.11 Å. An inspection of Scheme 1 shows that all these atoms are in the part purely described with the MM method, and the optimized IMOMM values are very close to the optimal bond distance for these types of atoms in the applied force field, which is 1.332 Å. A modification of the force field would undoubtedly correct this result. However, to keep the fairness of the test, introduction of experimental parameters in the calculation has been kept to a minimum.

Another discrepancy in the geometries appears in the Fe—N distance. This is more puzzling, because the distance oscillates between 2.016 Å for pure QM and 1.940 Å for the hybrid QM/MM, with the experimental value of 1.966 Å lying in between. Although the discrepancy of 0.05 Å with respect to the experimental number is in no case dramatic, this result could raise some concern on the accuracy of the description of the Fe—N bond in the IMOMM calculation. Furthermore, the overall agreement of the optimized geometries between them and with experiment could also be attributed to the steric constraints introduced by the presence of the porphyrin ring in the MM part of the calculation. In other words, one could still argue that the QM properties of the region around the metal atom are not well reproduced. Because of this, a more exigent test than comparison of optimized geometries was carried out.

The energy cost of the out of plane displacement of the metal atom was examined through both pure Becke3LYP and IMOMM(Becke3LYP:MM3) calculations. The iron atom was displaced within the symmetry axis of the system, keeping the rest of the geometry frozen at the respective optimized values. The energy cost of this type of movement will be ruled almost exclusively by electronic effects, i.e., by the binding of the Fe to the N atoms of the porphyrin ring. It will be, therefore, a good test of the quality of the description of this binding. Furthermore, it has a chemical interest in itself, because this type of movement of the metal atom seems to be a key factor in the behavior of a number of biological heme groups. The energy cost of the out of plane displacement of Fe in the 4-coordinate system computed with the two different methods is presented in Figure 2. The agreement between the two computational levels is excellent. The discrepancy between both calculations is always inferior to 1 kcal/mol, even with displacements as large as 0.5 Å, with a high energy cost of ca. 20 kcal/mol. It follows that the description of the Fe—N bond is essentially the same in both calculations, regardless of the fact that only part of the aromatic ring is treated quantum mechanically in the IMOMM calculation.

The 5-Coordinate [Fe(P)(Im)] System

Coordination of an imidazole ligand to the heme group leads to a 5-coordinate species with a square pyramidal geometry. This type of compounds are good biomimetic models of deoxymyoglobin and deoxyhemoglobin, the imidazole replacing

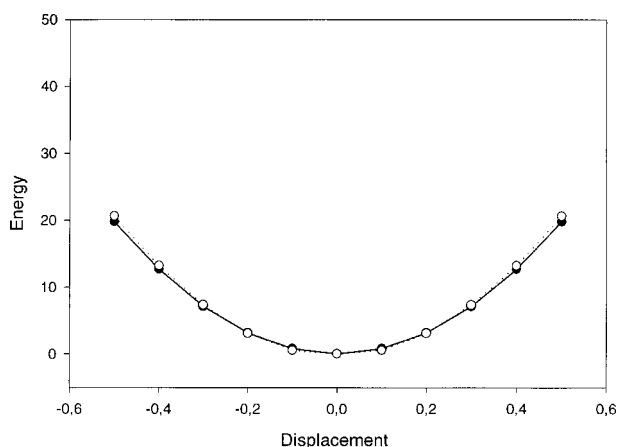


FIGURE 2. Energy cost (in kcal/mol) associated to the out-of-plane displacement (in Å) of the Fe atom in the 4-coordinate system. Solid line and solid circles correspond to the pure QM calculation, and dashed line and empty circles correspond to the hybrid QM/MM calculation.

the proximal histidine of the biological systems. The need to avoid both the dimerization and the formation of the 6-coordinate species with the two axial ligands poses serious restrictions on the nature of the porphyrins able to give this kind of complexes. For our comparison, we have chosen the species [Fe(Piv₂C₈)(1 – (Me)Im)] (Piv₂C₈ = $\alpha, \alpha, 5, 15$ -[2,2'-(octanediamido)diphenyl]- $\alpha, \alpha, 10, 20$ -bis(*o*-pivalamidophenyl)porphyrin) among the available structures.³⁸ This species has the advantage for our comparison of having 1-methylimidazole as axial ligand, in contrast with the more common 2-methylimidazole, which is more sterically demanding.

Unfortunately, neither for [Fe(Piv₂C₈)(1 – (Me)Im)] nor for other 5-coordinate derivatives of heme the electronic state is experimentally known. Electronic spectroscopy, magnetic susceptibility and Mössbauer measurements are conclusive in identifying it as high spin ($S = 2$, with four unpaired electrons on the iron atom), but are unable to find the molecular orbitals where the unpaired electrons are. This question is not trivial. The iron atom, formally Fe(II), has six valence electrons, to be placed in its *d* orbitals. The high-spin state means that four of the orbitals are singly occupied, and the remaining one is doubly occupied. Even after discarding the clearly antibonding d_{xy} and d_{z^2} orbitals, there are still three good candidates to be the doubly occupied orbital. These are $d_{x^2-y^2}$, d_{xz} , and d_{yz} , all of them nonbonding in the absence of π interactions.

Full *ab initio* geometry optimizations have been carried out on the $[\text{Fe}(\text{P})(\text{NH}=\text{CH}_2)]$ complex with the three different electronic states emerging from the double occupancy of each of these three atomic orbitals. The ground state happens to have two electrons in a linear combination of d_{xz} and d_{yz} , followed 1.8 kcal/mol above by the state with the other linear combination of these same orbitals doubly occupied. The excited state with the $d_{x^2-y^2}$ orbital doubly occupied has a higher relative energy of 2.2 kcal/mol. The close relationship between d_{xz} and d_{yz} could be expected *a priori*, because the equivalence between them is only broken by the π effects of the imidazole ligand. At any rate, our calculations predict that the valence β electron of iron will be placed in these axial orbitals, and not in the equatorial $d_{x^2-y^2}$ orbital.

Calculations were carried out on the ground state at the Becke3LYP and IMOMM(Becke3LYP:MM3) computational levels. The pure QM calculations were performed on the $[\text{Fe}(\text{P})(\text{NH}=\text{CH}_2)]$ model system, where the imine ligand tries to reproduce the electronic effects of imidazole. The hybrid QM/MM calculations were carried out on the $[\text{Fe}(\text{P})(1 - (\text{Me})\text{Im})]$ system. Selected geometrical parameters are collected in Table II. The optimized IMOMM structure is presented in Figure 3. Internal bond lengths and bond angles within the porphyrin ring follow exactly the same trends discussed above for 4-coordinate complexes, and are therefore not presented. It is, however, worth mentioning the RMS deviation from planarity of the 24 atoms of the porphyrin ring. The computed values at both the pure QM and the QM/MM levels are near 0.05 Å, in sharp contrast with the value of 0.00 Å obtained in the 4-coordinate system. Though the computed values are still far from the experimental value of 0.131 Å, this discrepancy is likely related to the presence of the large substituents in the porphyrin. Our calculations confirm at any rate that there is an intrinsic deviation from planarity associated to the coordination of a fifth ligand. The observed distortion points toward a C_{4v} arrangement.

The average distances between the metal atom and the nitrogen atoms in the porphyrin ring show the same type of dispersion already observed in the 4-coordinate complex, with the experimental value lying between the pure Becke3LYP and IMOMM(Becke3LYP:MM3) values. All the $\text{Fe}-\text{N}_p$ distances in this 5-coordinate complex are longer by ca. 0.1 Å than those in their 4-coordinate counterpart. This is fully consistent with the shift from low spin to high spin in the metal involving the placement of one electron in the antibonding d_{xy} orbital.

TABLE II. Selected Geometrical Parameters (Å and Degrees) from the Geometry Optimization of $[\text{Fe}(\text{P})(\text{NH}=\text{CH}_2)]$ with the Pure Becke3LYP and of $[\text{Fe}(\text{P})(1 - (\text{Me})\text{Im})]$ with the IMOMM(Becke3LYP:MM3) Methods.

	Experiment	Pure QM	QM/MM
$\text{Fe}-\text{N}_p^a$	2.074	2.101	2.029
$\text{Fe}-\text{N14}$	2.134	2.252	2.233
$\text{N14}-\text{C15}$	1.35	1.279	1.299
$\text{N14}-\text{C16}$	1.25	— ^b	1.414 ^c
$\text{Fe}-\text{N14}-\text{C15}$	127.	126.1	136.8
$\text{Fe}-\text{N14}-\text{C16}$	120.	120.6	122.6
$\text{N2}-\text{Fe}-\text{N14}-\text{C15}$	−54.	−90.0	−43.2
Fe-plane^c	−0.342	−0.316	−0.411
RMS displ.^d	0.131	0.043	0.054

Experimental data on the $[\text{Fe}(\text{Piv}_2\text{C}_8)(1 - (\text{Me})\text{Im})]$ system are also provided for comparison. Labeling of atoms is that from Scheme 1.

^a Average values.

^b Corresponds to N—H in this calculation.

^c Frozen in calculation.

^d Distance of the iron atom to the mean plane of the porphyrin ring.

^e Average out of plane displacement of the 24 atoms of the porphyrin ring.

Most data in Table II focus on the description of the imidazole ligand. The $\text{Fe}-\text{N}_{\text{im}}$ distance is longer by ca. 0.10 Å in calculation than in experiment. We are not able to explain the origin of this discrepancy. At any rate, both computed values are very close to each other (2.252 Å vs. 2.233 Å), proving that the use of the QM/MM method does not introduce any error. Overall agreement in the geometrical parameters is correct. Moreover, one has to take with some suspicion the X-ray parameters of the imidazole ligand, which would make the $\text{N}=\text{C}$ double

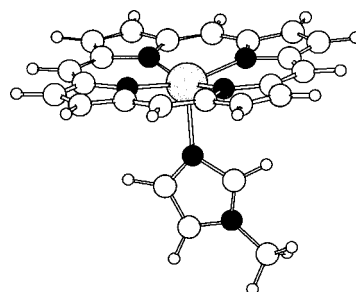


FIGURE 3. Optimized IMOMM(Becke3LYP:MM3) geometry of the 5-coordinate complex $[\text{Fe}(\text{P})(1 - (\text{Me})\text{Im})]$.

bond N14—C15 of imidazole longer than the N—C single bond N14—C16.

The sharper discrepancy shown in Table II concerns the N2—Fe—N14—C15 dihedral angle. This angle measures the rotation around the Fe—N14 single bond, and rules the placement of the imidazole plane with respect to the porphyrin ring. Its sign is arbitrary, because the *x* and *y* directions are equivalent in absence of axial ligand. We have chosen a negative sign for consistence with data on the 6-coordinate complex presented below. An angle of -90.0 degrees (like in the pure QM calculation) means that the imidazole plane is eclipsing one of the Fe—N_p bonds, while an angle of -45.0 degrees (close to the -43.2° found in the QM/MM calculation) indicates a staggered orientation of the imidazole with respect to the Fe—N_p bonds. The two computed values are, therefore, just opposite, with the experimental value (-54°) lying in between, although closer to the QM/MM value. The importance of this large discrepancy between different values is, however, arguable, because there is also a large dispersion in different experimental 5-coordinate derivatives of heme,⁸ as well as in experimental reports of deoxymyoglobin and deoxyhemoglobin. It seems, therefore, that rotation around this single bond has a very low barrier. We are planning to further analyze this question, but preliminary calculations confirm a very low barrier of 1.5 kcal/mol in pure QM calculations on [Fe(P)(Im)].

A final number to mention from Table II is the displacement of the iron atom out of the 24-atom porphyrin plane. This is one of the most important parameters, because it seems to play a critical role in the cooperativity of hemoglobin involved in the allosteric transition. In this case, agreement between experiment and calculation is good, with values of -0.342 , -0.316 , and -0.411 Å, for the experiment and the two calculations, respectively. The negative value corresponds to the fact that the displacement is towards the imidazole, and away from the empty site.

The energy cost of the out-of-plane displacement of the metal atom was also examined in the 5-coordinate system. In this case, the displacement was carried out in the direction of the Fe—N_{im} bond. Moreover, the axial ligand was moved together with the metal, with the Fe—N_{im} distance being frozen at the corresponding optimized value. The results are shown in Figure 4. Again, agreement is very good, with the curves computed through pure QM and through hybrid QM/MM methods lying very close to each other. It is worth mention-

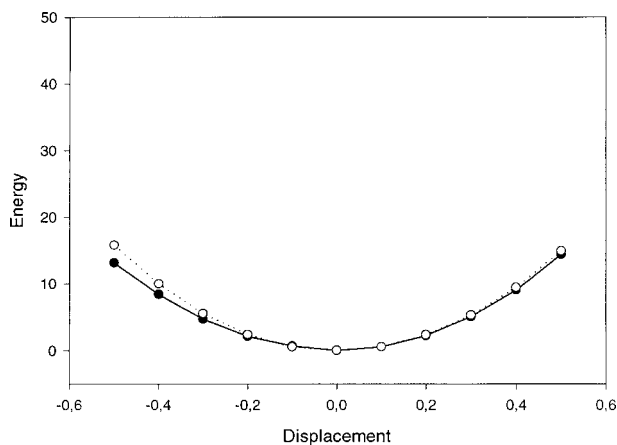


FIGURE 4. Energy cost (in kcal/mol) associated to the out-of-plane displacement (in Å) of the Fe atom around its equilibrium position in the 5-coordinate system. Solid line and solid circles correspond to the pure QM calculation, and dashed line and empty circles correspond to the hybrid QM/MM calculation.

ing also that the out-of-plane displacement is less energy demanding in the 5-coordinate system (ca. 5 kcal/mol for a move of 0.3 Å) than it was for the 4-coordinate system (ca. 10 kcal/mol for the same move of 0.3 Å). This difference between the two systems is well reproduced in the IMOMM calculation.

An additional result that can be obtained from the calculations presented in this section and the previous one is an estimation of the binding energy of the axial imidazole ligand to the 4-coordinate complex. This can be done by direct subtraction of the energy of the separated 4-coordinate plus imidazole fragments from that of the 5-coordinate. The result of this operation presents a rather poor agreement between the two calculations, with values of -2.7 kcal/mol for the pure QM calculation and $+8.4$ kcal/mol for the QM/MM calculation. These numbers should not be taken as direct estimations of the binding energy. Other, more elaborate, schemes than this simple subtraction have been suggested to compute binding energies in this type of systems,¹⁵ and a number of possibly significant corrections, like basis set superposition error, have been neglected. However, the systematic errors should be the same in both calculations, and one would expect a more similar result. Our conclusion is that the hybrid QM/MM method applied, while performing well in predicting the equilibrium geometry and relative distortion energies from it, has a poorer performance in predicting the binding energy of the imidazole ligand.

Finally, a supplementary set of pure QM calculations on the 5-coordinate systems was carried out on [Fe(P)(Im)] and [Fe(P)(1 – (Me)Im)] to check for possible inaccuracies associated with the use of the [Fe(P)(NH=CH₂)] model system. The optimized structures with the imidazole and methylimidazole ligands were found to be similar to those with the imine model. Discrepancies in bond distances within the Fe(P) unit were always smaller than 0.01 Å. The coordination of the axial ligand was, logically, more affected, but changes were also small. The Fe—N14 distance (2.252 Å) was 2.226 Å with imidazole, and 2.213 Å with methylimidazole. The out-of-plane displacement was also affected, with values of –0.363 Å for imidazole and –0.376 Å for methylimidazole (to be compared with –0.316 Å for the imine model). These small changes bring actually the QM results closer to the QM/MM ones (Table II), and do not explain in any case the discrepancies with the X-ray structures.

The 6-Coordinate [Fe(P)(Im)(O₂)] System

Coordination of a dioxygen molecule to the 5-coordinate species derivated from heme discussed in the previous section leads to 6-coordinate species with an octahedral geometry. These types of compounds are the biomimetic forms of oxymyoglobin and oxyhemoglobin. Despite their large interest, X-ray data have been reported only on two complexes with this stoichiometry: [Fe(T_{piv}PP)(1 – (Me)Im)(O₂)],³⁹ and [Fe(T_{piv}PP)(2 – (Me)Im)(O₂)].⁴⁰ Both complexes are quite similar, sharing the same porphyrin T_{piv}PP, which is *meso*-tetrakis($\alpha, \alpha, \alpha, \alpha$ -*p*-pivalamidophenyl)porphyrin. We have chosen for comparison the [Fe(T_{piv}PP)(1 – (Me)Im)(O₂)] complex, containing the less sterically demanding 1-methylimidazole ligand.

A preliminary study²⁴ on the geometry optimization of this compound with the IMOMM (Becke3LYP:MM3) method has already been published, albeit with a slightly different basis set in the QM part. The change in the basis set introduces only minor modifications in the geometry, and the results are only briefly discussed here for completion of the methodological analysis. The spin state of this system has a singlet nature, and we have assumed in our calculations, as the authors of other theoretical studies,²³ that the three doubly occupied *d* orbitals of Fe are those corresponding to the *t*_{2g} set, namely *d*_{x²–y²}, *d*_{xz}, and *d*_{yz}. This is actually the elec-

tronic structure closest to the ground state obtained in CASSCF calculations with a large active space.

Recent DFT calculations by the group of Parrinello¹⁶ indicate that the ground state of this system is an open-shell singlet, where one electron of one of the two doubly occupied *d* _{π} orbitals of Fe is transferred to the π^* orbital of O₂, resulting in a Fe^{III}—O₂[–] system. Our attempts to reproduce this result, obtained with a plane wave algorithm,¹⁶ with the more conventional algorithms of the Gaussian program²⁵ through the use of a spin-contaminated broken symmetry solutions have lead to structures where the dioxygen unit dissociates from the metal center. We attribute the discrepancy in results to the difference in the computational method. We are aware of the potential relevance of this controversy to the chemistry of the system. However, because the difference between the open-shell and close-singlets is concentrated in the Fe—O₂ subunit, within the QM region, we consider it does not affect the validity of the introduction of a QM/MM partition within the porphyrin ring, which is the subject of this article.

Calculations were carried out on the closed-shell singlet electronic state at the Becke3LYP and IMOMM(Becke3LYP:MM3) computational levels. The pure QM calculations were performed on the [Fe(P)(NH=CH₂)(O₂)] system, and hybrid QM/MM calculations were carried out on the [Fe(P)(1 – (Me)Im)(O₂)] system. Selected geometrical parameters are collected in Table III, and the optimized IMOMM structure is presented in Figure 5. A number of the results of the geometry optimization confirm trends already observed on the calculations on the other two systems. Internal geometrical parameters of the porphyrin ring are practically the same, and are not included because of the little changes with respect from those of the 4-coordinate and 5-coordinate system. The computed RMS deviation of the 24 atoms of the porphyrin ring from the plane is of the same order than that of the 5-coordinate system. The experimental Fe—N_p distance is again shorter than that obtained in the QM calculation and longer than that obtained in the QM/MM calculation. The experimental trend of having this Fe—N_p distance in the 6-coordinate system slightly longer than in the 4-coordinate system and quite shorter than in the 5-coordinate system is, nevertheless, well reproduced by both levels of calculation. A description of the coordination of imidazole presents similar problems to those already observed in the 5-coordinate system. The shortening of the Fe—N14 distance associated to the loss of one α electron from the antibonding *d*_{z²} orbital is, nevertheless,

TABLE III.
Selected Geometrical Parameters (Å and Degrees)
from the Geometry Optimization of
[Fe(P)(NH=CH₂)(O₂)] with the Pure Becke3LYP and
of [Fe(P)(1 – (Me)Im)(O₂)] with the
IMOMM(Becke3LYP:MM3) Methods.

	Experiment	Pure QM	QM/MM
Fe—N _p ^a	1.978	2.035	1.949
Fe—N14	2.070	2.050	2.167
Fe—O12	1.746	1.757	1.759
O12—O13	1.163	1.268	1.286
Fe—O12—O13	129.4	121.1	117.0
O12—Fe—N14	180.0	175.8	179.4
N2—Fe—N14—C15	159.5	177.9	133.0
N2—Fe—O12—O13	−42.4	−44.6	−45.9
Fe-plane ^b	+0.014	+0.019	+0.061
RMS displ. ^c	0.072	0.044	0.033

Experimental data on the [Fe(T_{div}PP)(1 – (Me)Im)(O₂)] system are also provided for comparison. Labeling of atoms is that from Scheme 1.

^a Average values.

^b Distance of the iron atom to the mean plane of the porphyrin ring.

^c Average out of plane displacement of the 24 atoms of the porphyrin ring.

well reproduced in the calculation. The values for the dihedral angle ruling the rotation around the Fe—N14 bond are also very different. In this case, the best agreement with experiment is given by the pure QM calculation.

Parameters concerning the coordination of O₂ and the out of plane displacement of Fe, which are probably the most critical for the biochemical activity of hemoglobin, are very well reproduced. The two computed values for the Fe—O distance, 1.757 and 1.759 Å, are practically identical to the experimental value of 1.746 Å. The two computed values

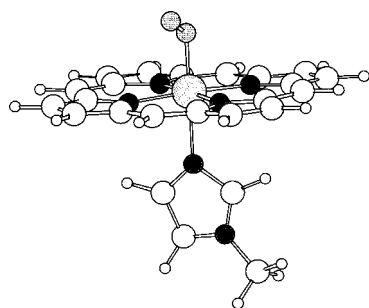


FIGURE 5. Optimized IMOMM(Becke3LYP:MM3) geometry of the 6-coordinate complex [Fe(P)(1 – (Me)Im)(O₂)].

(1.268, 1.286 Å) for the O—O distance are close to each other, but far from the experimental report of 1.163 Å. However, the experimental value (even shorter than the value of 1.21 Å for free oxygen!) is suspect, because of the disorder on the placement of the second oxygen within the crystal, as admitted by the authors of the X-ray experiment themselves.³⁹ A similar reasoning can be used for the Fe—O—O bond angles, which are, nevertheless, in all cases indicative of a bent η^1 coordination mode, where only one of the oxygen atoms is directly attached to the metal. The orientation of the Fe—O—O plane with respect to the Fe—N_p axis is staggered, in both calculations (−44.6°, −45.9°) and in the experiment (−42.4°). Finally, the iron atom is computed to lie in the energy minimum very close to the porphyrin plane, with a deviation smaller than 0.1 Å, always towards the oxygen. This is, again, in agreement with the experiment, and very different from the behavior of the 5-coordinate system.

To finish the study on the 6-coordinate systems, the energy cost of the out-of-plane displacement of the metal atom was also studied. In this case, the iron atom was displaced also along the Fe—N_{im} direction, and it was moved together with the two axial ligands. The results are presented in Figure 6. Although agreement is not as perfect as in the other two cases previously analyzed, the overall similarity between the Becke3LYP and the IMOMM(Becke3LYP:MM3) curves is still remarkable. The out-of-plane displacement is more energetically costly in this case of the 6-coordinate

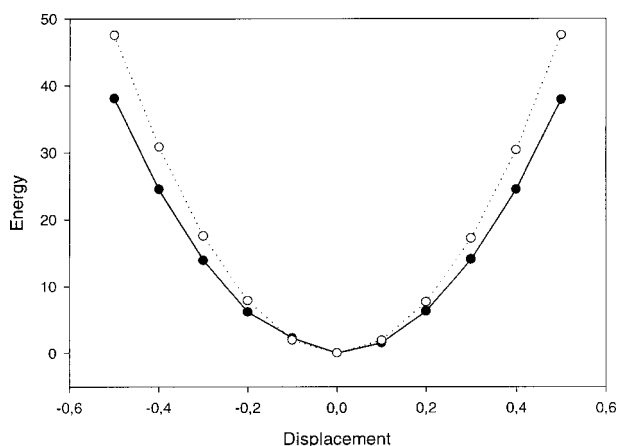


FIGURE 6. Energy cost (in kcal/mol) associated to the out-of-plane displacement (in Å) of the Fe atom around its position of equilibrium in the 6-coordinate system. Solid line and solid circles correspond to the pure QM calculation, and dashed line and empty circles correspond to the hybrid QM/MM calculation.

species, with an increase of ca. 15 kcal/mol for a move of 0.3 Å. This behavior, which may have interesting implications on the reactivity of the system, is well reproduced in the cheaper hybrid calculation.

Finally, the binding energy of dioxygen to the 5-coordinate system was evaluated through subtraction of the energy of the separated fragments from that of the 6-coordinate system. The resulting values were +9.5 and +5.6 kcal/mol for the pure QM and the QM/MM calculations, respectively. Although agreement is better than for the binding energy of imidazole, this magnitude remains by far the worst reproduced by the QM/MM calculation among those considered.

QM Calculations on Small Model Systems

Results in the previous sections prove that the IMOMM calculations provide results of comparable quality to those of much more expensive pure QM calculations. In fact, the cost of the IMOMM calculation is equivalent to that of a pure QM calculation on the QM region of the hybrid calculation, because the computational cost of the MM part is in practice neglectable. It is, therefore, interesting to evaluate the quality of a pure QM calculation on the model system used for the QM region of the IMOMM calculation. If the result were not different from that of the IMOMM calculation, the introduction of the MM region would be useless.

A pure QM calculation was carried out on the $[\text{Fe}(\text{NH}(\text{CH}_3)\text{NH}_2)_2(\text{Im})]$ system. Although some parameters (namely, the Fe—N distances) were similar to those obtained in the IMOMM calculations on the 5-coordinate systems, very substantial differences appeared in two parameters. These were the out-of-plane displacement of the metal atom and the RMS displacement of atoms in the porphyrin ring. The out-of-plane displacement of Fe, which has values around 0.3–0.4 Å for $[\text{Fe}(\text{P})(\text{Im})]$ systems (Table II), climbs to a value of 0.652 Å in this pure QM calculation on the model system. The RMS displacement increases even more substantially, from values below 0.1 Å for $[\text{Fe}(\text{P})(\text{Im})]$ to a value of 0.331 Å in $[\text{Fe}(\text{NH}(\text{CH}_3)\text{NH}_2)_2(\text{Im})]$. The qualitative difference between those distances is certainly in the loss of planarity of the remains of the porphyrin ring in the pure QM calculation. In this new calculation they rearrange themselves in two different planes, one for each fragment, with no correlation between them. The distortions from planarity, which have

been postulated as important factors in the biological properties of heme groups,^{36, 41, 42} will, therefore, be poorly reproduced by pure QM calculations on small model systems.

Methodological Discussion on the QM Calculations

The quantum mechanics part absorbs most of the computer effort of the IMOMM calculation. Moreover, available QM methods come at very different computational prices, the most expensive usually providing the highest quality. It is, therefore, important to choose the cheapest QM method able to provide the desired quality. This section contains a brief discussion on the reasons justifying the choice of the QM methods applied in the rest of the work. In particular, basis sets of different qualities are tested; and the unrestricted Becke3LYP method is confronted with the cheaper unrestricted Hartree–Fock method.

Tables IV and V present the results of the application of different basis sets to the calculation of the 4-coordinate system at the IMOMM(UHF:MM3) and IMOMM(Becke3LYP:MM3) levels. Basis sets II to VI, defined in the computational details, are numbered by order of increasing quality. Basis set I, the one used in the rest of the article, would involve a similar computational effort to that of basis set IV. Tables IV and V show that the basis set has little effect on the parameters under discussion. The most sensitive geometrical parameter is the Fe—N2 distance with a dispersion of 0.039 Å at the IMOMM(UHF:MM3) level, and of 0.029 Å at the IMOMM(Becke3LYP:MM3) level. Nevertheless, most of this dispersion is related to the presence of the smaller basis set II, minimal for all atoms but the metal. If this basis set II is taken out, the dispersion in Fe—N2 distances comes down to 0.007 and 0.005 Å, respectively. That is, all the five better basis sets yield results identical to the 1/10 of an Å. The energy cost for the out of plane displacement of iron seems to be a little more sensitive to the basis set, albeit exaggerated because of the large displacement of 0.5 Å being considered. Again, basis set II stands out from the rest, and can be directly discarded. The other five basis sets can be divided in this case in two groups. Basis sets III and IV are very close to each other (dispersions of 0.4 and 0.1 kcal/mol, at the UHF and UBecke3LYP levels); and more distanced from I, V, and VI, which are also grouped together (dispersions of 0.2 at both computational levels). The key factor seems to be the basis set at

TABLE IV. Selected Results (Å, Degrees, and kcal/mol) from the IMOMM(UHF:MM3) Geometry Optimization of the 4-Coordinate [Fe(P)] with Different Basis Sets.

	I	II	III	IV	V	VI
Fe—N2	1.981	1.947	1.979	1.986	1.986	1.982
N2—C6	1.341	1.348	1.344	1.329	1.321	1.316
C6—C7	1.387	1.386	1.384	1.389	1.393	1.392
Fe—N2—C6	127.6	127.9	127.6	127.5	127.4	127.5
Fe—N2—C20	125.8	126.8	125.4	125.3	125.7	125.5
N2—Fe—N3	89.2	89.6	89.0	88.9	89.2	89.0
N2—Fe—N4	90.8	90.4	91.0	91.0	90.8	91.0
Energy cost	16.3	22.9	18.7	19.1	16.5	16.4

The energy cost presented corresponds to the displacement of the Fe atom 0.5 Å out of the plane of the molecule. Labeling of atoms is that from Scheme 1, and basis sets are defined in the Computational Details.

nitrogen, which is 6-31G for III, IV; and 6-31G(d) for I, V, and VI. The basis set at carbon and hydrogen, further away from the metal, has a much smaller effect. Because of this, we chose for the rest of the calculations basis set I, which is the cheapest one in the group providing the most accurate results.

Comparison between Tables IV and V shows that the results are more affected by the change of the method from UHF to UBecke3LYP than they are by the change of basis set within one method. Nevertheless, they are still quite similar. One could, therefore, be tempted to choose the cheaper UHF method for the QM part of all calculations. We certainly tried this strategy, with the result of an extremely poor description of the 6-coordinate system. What happens in the UHF calculation on [Fe(P)(NHCH₂)(O₂)] is that the Fe—O oxygen distance increases to val-

ues above 3 Å (to be compared with the experimental or Becke3LYP value of ca. 1.75 Å). This means that the oxygen molecule does not bind to the complex at the UHF level. This failure is very probably related to the fact that this bond has an important component of backdonation from the metal center to the dioxygen unit, and backdonation is largely underestimated in the absence of electron correlation. It is worth mentioning that UHF calculations with a minimal basis set for oxygen provide a “pseudobonding” distance of ca. 2.2 Å. We relate this result to the basis set superposition error associated to the unbalance between the basis sets on the metal and on the oxygen. At any rate, the complete failure of the UHF method in the description of the 6-coordinate systems prompted us to discard it, and to use UBecke3LYP as the computational method throughout the article.

TABLE V. Selected Results (Å, Degrees, and kcal/mol) from the IMOMM(Becke3LYP:MM3) Geometry Optimization of the 4-Coordinate [Fe(P)] with Different Basis Sets.

	I	II	III	IV	V	VI
Fe—N2	1.940	1.916	1.944	1.945	1.944	1.945
N2—C6	1.362	1.386	1.366	1.355	1.348	1.348
C6—C7	1.401	1.401	1.399	1.391	1.393	1.393
Fe—N2—C6	128.7	129.0	128.5	128.0	128.0	127.9
Fe—N2—C20	126.7	127.6	126.5	126.7	126.4	127.0
N2—Fe—N3	89.5	89.8	89.5	89.7	89.8	89.9
N2—Fe—N4	90.5	90.2	90.5	90.3	90.2	90.1
Energy cost	19.8	23.5	21.3	21.4	20.0	19.9

The energy cost presented corresponds to the displacement of the Fe atom 0.5 Å out of the plane of the molecule. Labeling of atoms is that from Scheme 1, and basis sets are defined in the Computational Details.

Concluding Remarks

IMOMM(Becke3LYP:MM3) calculations on the 4-coordinate [Fe(P)], the 5-coordinate [Fe(P)(1 – (Me)Im)], and the 6-coordinate [Fe(P)(1 – (Me)Im)(O₂)] systems give results that are in good agreement with full QM calculations on analogous systems. Agreement is especially close in the case of optimized geometrical parameters and the energy cost of displacement of the iron atom out of the plane of the porphyrin ring. Discrepancies are more significant in the case of the binding energy of the axial ligands to the heme group. Optimized geometries are also close to experimental X-ray geometries, and the differences are likely associated to the experimental existence of bulky substituents in the porphyrin and of crystal packing effects, which are absent from the gas phase calculations.

The use of an MM description for a significant part of the heme group porphyrin ring, therefore, brings a major reduction in computer effort with a minor reduction of quality in the results—very minor in some properties. The application of hybrid QM/MM methods to systems containing heme groups thus appears as a very promising venue for the theoretical study of the structure and reactivity of these biologically relevant systems.

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